



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 5/06, 5/22, 9/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/50392</b> <b>(43) International Publication Date:</b> 7 October 1999 (07.10.99)
<b>(21) International Application Number:</b> PCT/US99/06898 <b>(22) International Filing Date:</b> 30 March 1999 (30.03.99)  <b>(30) Priority Data:</b> 60/112,006 31 March 1998 (31.03.98) US  <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application</b> US 60/112,006 (CON) Filed on 31 March 1998 (31.03.98)  <b>(71) Applicant (for all designated States except US):</b> GERON CORPORATION [US/US]; 230 Constitution Drive, Menlo Park, CA 94025 (US).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> GAETA, Federico, C., A. [US/US]; 1589 Carole Way, Redwood City, CA 94061 (US).  <b>(74) Agents:</b> APPLE, Randolph, T. et al.; Townsend and Townsend and Crew LLP, 8th floor, Two Embarcadero Center, San Francisco, CA 94111-3834 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> METHODS AND COMPOSITIONS FOR ELICITING AN IMMUNE RESPONSE TO A TELOMERASE ANTIGEN  <b>(57) Abstract</b> <p>The invention provides a method of activating a T lymphocyte by contacting the T lymphocyte with a dendritic cell (DC) that presents a telomerase reverse transcriptase (TRT) peptide in the context of a MHC class I or MHC class II molecule. The DC may be pulsed with a TRT polypeptide or may comprise a recombinant polynucleotide encoding a TRT such as hTRT. The invention also provides DCs comprising a recombinant TRT polynucleotide. The methods and compositions of the invention are used in prevention and treatment of cancers and other cell proliferation diseases or conditions.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LJ	Liechtenstein	SD	Sudan		
DE	Germany	LR	Sri Lanka	SE	Sweden		
DK	Denmark		Liberia	SG	Singapore		
EE	Estonia						

## METHODS AND COMPOSITIONS FOR ELICITING AN IMMUNE RESPONSE TO A TELOMERASE ANTIGEN

5

### CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of U.S. Patent Application Serial Number 08/974,549, filed November 19, 1997, and a continuation-in-part of U.S. Patent Application Serial Number 08/974,584, filed November 19, 1997, both of which are continuation-in-part applications of U.S. Patent Application Serial Number 08/915,503, U.S. Patent Application Serial Number 08/912,951, and U.S. Patent Application Serial Number 08/911,312, all filed August 14, 1997. This application also claims the benefit of Patent Convention Treaty Patent Application Serial No.: PCT/US97/17885 and to Patent Convention Treaty Patent Application Serial No.: PCT/US97/17618, both filed on October 1, 1997. Each of the aforementioned applications is explicitly incorporated herein by reference in its entirety and for all purposes.

### FIELD OF THE INVENTION

The present invention is related to the catalytic protein subunit of human telomerase. The invention provides methods and compositions relating to medicine, immunology, and molecular biology.

### BACKGROUND OF THE INVENTION

The following discussion is intended to introduce the field of the present invention to the reader. The citation of references in this section should not be construed as an admission of prior invention.

The telomerase ribonucleoprotein complex is a specialized polymerase that maintains telomeres, the specialized structures at the ends of eukaryotic chromosomes. The length and integrity of telomeres in a cell is correlated with the entry of the cell into a senescent stage (*i.e.*, loss of proliferative capacity), or, alternatively, the ability of a cell to escape senescence. Of particular interest is the correlation observed between telomerase

activity in human and other mammalian cells and the development of a neoplastic phenotype. For example, telomerase activity is detected in immortal cell lines and a diverse set of tumor tissues, but is not detected (*i.e.*, was absent or below the assay threshold) in normal somatic cell cultures or normal tissues adjacent to a tumor (see, U.S. Patents Nos. 5,629,154; 5,489,508; 5,648,215; and 5,639,613; also see, Morin, 1989, *Cell* 59: 521; Shay and Bacchetti, 1997, *Eur. J. Cancer* 33:787; Kim et al., 1994, *Science* 266:2011; Counter et al., 1992, *EMBO J.* 11:1921; Counter et al., 1994, *Proc. Natl. Acad. Sci. U.S.A.* 91, 2900; Counter et al., 1994, *J. Virol.* 68:3410). Moreover, a correlation between the level of telomerase activity in a tumor and the likely clinical outcome of the patient has been reported (e.g., U.S. Patent No. 5,639,613; Langford et al., 1997, *Hum. Pathol.* 28:416).

Thus, telomerase plays an important role in the control of cell proliferation and in tumorigenesis. For these and other reasons, human telomerase is an ideal target for preventing and treating human diseases relating to cellular proliferation and senescence, such as cancer. The present invention provides immunological methods for preventing and treating these and other diseases in humans and nonhuman animals.

### BRIEF SUMMARY OF THE INVENTION

In one aspect, the present invention provides a method of activating a T lymphocyte by contacting the T lymphocyte with a dendritic cell that expresses a telomerase reverse transcriptase (TRT) polypeptide encoded by a recombinant nucleic acid. In various embodiments of the invention, the TRT polypeptide is a human TRT (hTRT) polypeptide, and may have the sequence set forth in Figure 1, or may have a subsequence thereof. In one embodiment, the hTRT polypeptide is full-length. In one embodiment, the dendritic cell is a human cell. The dendritic cell may contact the T lymphocyte *in vivo* or *in vitro*.

In a related aspect, the invention provides a recombinant dendritic cell which comprises a recombinant TRT expression cassette. In one embodiment, the recombinant expression cassette is transduced into a stem cell, and the stem cell is then differentiated into the dendritic cell. In one embodiment, the stem cell is differentiated *in vitro*. The invention also provides a pharmaceutical composition comprising the

aforementioned dendritic cell and a pharmaceutically acceptable carrier.

In another aspect, the invention provides a method of eliciting an immune response in a human patient by (a) obtaining human dendritic cells, (b) transducing a TRT expression cassette into the cells so that they are capable of  
5 expressing a hTRT polypeptide, and (c) administering the cells to the human patient. In certain embodiments, the dendritic cells are isolated from the human patient to which they are administered, and/or are obtained from hematopoietic precursor cells.

In yet another aspect, the invention provides a method of eliciting an immune response in a human patient by (a) obtaining human dendritic cells, (b) pulsing  
10 the cells with a hTRT antigen, and (c) administering the cells pulsed with the hTRT antigen to the human patient. In certain embodiments, the dendritic cells are isolated from the human patient to which they are administered, and/or are obtained from hematopoietic precursor cells. In one embodiment the cells are pulsed with one or more hTRT antigenic peptides that are less than 50 amino acid residues in length.

15 In another aspect, the invention provides a method for identifying a cell expressing hTRT. According to the method, a dendritic cell is transduced with a recombinant expression cassette comprising a nucleic acid encoding a hTRT polypeptide; a T lymphocyte is contacted with the transduced dendritic cell, thereby providing an activated T lymphocyte; and a target cell is contacted with the activated T  
20 lymphocyte. The effect of the activated T lymphocyte on the target cell is then monitored.

### DESCRIPTION OF THE FIGURES

Figure 1 shows the amino acid sequence (in the one-letter code) of a  
25 1132-residue hTRT protein.

Figure 2 shows a nucleic acid sequence of a cDNA encoding the hTRT protein shown in Figure 1.

## DETAILED DESCRIPTION OF THE INVENTION

### I. DEFINITIONS

As used herein, "telomerase reverse transcriptase" (TRT) refers to the catalytic protein subunit of the telomerase ribonucleoprotein particle. TRT from humans has been characterized, human TRT (hTRT) genomic and cDNA sequences have been cloned and their sequences determined. See, e.g., Nakamura et al., 1997, *Science* 277:955 and copending U.S. patent applications serial nos 08/912,951 and 08/974,549. The sequence of a full-length native hTRT has been deposited in GenBank (Accession No. AF015950), and plasmid and phage vectors having hTRT coding sequences have been deposited with the American Type Culture Collection, Rockville, Maryland (accession numbers 209024, 209016, and 98505). Nonhuman TRT polypeptides (i.e., hTRT homologs) are described in copending U.S. patent applications serial nos. 08/974,584, 08/974,549, 08/915,503, 08/912,951. hTRT polypeptides that may be used in the present invention include, in addition to the hTRT polypeptides having amino acid sequences described in the aforecited references and deposits, other naturally occurring variants, including allelic variants and processing variants. As described in detail in the copending patent applications (e.g., U.S.S.N. 08/912,951 and 08/974,549), it is well within the ability of one of skill to obtain naturally occurring hTRT variants or derivatives, and non-human homologs using the above-mentioned sequences and reagents and using, e.g., standard molecular biological techniques. In certain embodiments of the invention, a hTRT polypeptide or variant (or gene or cDNA encoding such a variant) characteristic of a particular subject (e.g., expressed in tumor cells from the subject) or characteristic of a particular tumor type is used. Such tumor or subject-specific hTRT can be obtained using standard methods, e.g., cloning using hTRT PCR primers, purification using anti-hTRT antibodies, and other techniques. Exemplary uses of hTRT polypeptides and polynucleotides, and nonhuman homologs, are described in additional detail in U.S. Patent Application Serial Numbers 08/974,549 (filed November 19, 1997), 08/974,584 (filed November 19, 1997), 08/915,503 (filed August 14, 1997), 08/912,951 (filed August 14, 1997), 08/911,312 (filed August 14, 1997), 08/854,050 (filed May 9, 1997), 08/851,843 (filed May 6, 1997), 08/846,017 (filed April 25, 1997), 08/844,419 (filed April 18, 1996), 08/724,643 (filed October 1, 1996), and 08/979,742 (filed November 26, 1997). Each of

the aforementioned patent applications is explicitly incorporated herein by reference in its entirety and for all purposes.

5 A "dendritic cell" (DC) is an antigen presenting cell (APC) with a characteristic morphology including lamellipodia extending from the dendritic cell body in several directions. Several phenotypic criteria are also typical, including high levels of MHC molecules and costimulatory molecules, a lack of markers specific for granulocytes, NK cells, B lymphocytes, and T lymphocytes, but can vary depending on the source of the dendritic cell. DCs are able to initiate antigen specific primary T lymphocyte  
10 responses *in vitro* and *in vivo*, and direct a strong mixed leukocyte reaction (MLR) compared to peripheral blood leukocytes, splenocytes, B cells and monocytes. DCs can be derived from a hematopoietic stem cell, e.g., a CD34<sup>+</sup> precursor cell. Dendritic cells are described generally by Steinman, 1991, *Annu Rev Immunol.* 9:271-96.

15 A cell is "transduced" with a selected nucleic acid when the nucleic acid is translocated into the cell. A cell is "stably transduced" with a selected nucleic acid when the selected nucleic acid is replicated and passed on to progeny cells. A cell is "transformed" with a selected nucleic acid when the selected nucleic acid is integrated into the cell's genome.

20 A "TRT expression cassette" is a nucleic acid construct, generated recombinantly or synthetically, that includes a nucleic acid encoding a TRT polypeptide or fragment, and a promoter. In some embodiments, the expression cassette also includes other genetic elements, e.g., an origin of replication, and/or chromosome integration  
25 elements such as retroviral LTRs. The TRT expression cassette may be plasmid, virus genome, nucleic acid fragment, or the like.

The term "recombinant" has its usual meaning in the art. Thus, a recombinant polynucleotide is a polynucleotide synthesized or otherwise manipulated *in*  
30 *vitro* (e.g., using molecular biological techniques), and is typically linked to sequence (e.g., a heterologous promoter, vector sequence or other sequence) with which it is not normally

linked in nature and/or comprises mutations (e.g., deletions) compared to naturally occurring forms; a "recombinant polypeptide" is a polypeptide that is produced by expression of a recombinant nucleotide; and a "recombinant cell" is a cell comprising a recombinant polynucleotide.

5

The terms "activation" or "activating," when used in reference to a T lymphocyte, has the ordinary meaning in the art of immunology and refers to characteristic changes (e.g., calcium ion influx, tyrosine kinase activation) that follow ligand-receptor interactions between a T lymphocyte and antigen presenting cell. T cell activation  
10 ordinarily results in clonal expansion of antigen-reactive T lymphocytes.

## II. INTRODUCTION

The methods and reagents of the invention are useful for eliciting an *in vivo* immune response to telomerase, a telomerase protein, and/or a cell expressing telomerase  
15 or a telomerase protein. In particular, the methods and reagents of the invention are used to treat or prevent diseases or conditions related to cell proliferation, such as cancers.

In humans and other mammals, many cells that proliferate indefinitely, such as cancer cells, are characterized by the expression of the endogenous telomerase reverse transcriptase (TRT) gene and the presence of detectable telomerase activity. In contrast,  
20 most normal somatic cells in mammals have no or very low levels of TRT expression. Thus, by eliciting a specific immune response to TRT or to TRT-expressing cells, it is possible to selectively target proliferating cells for immunological destruction. Reagents, such as the dendritic cells described *infra*, that induce differentiation and proliferation of T lymphocytes that specifically target telomerase expressing cells may be used for the  
25 prevention or treatment of cancer. Induction of an anti-TRT immune response in a human or nonhuman subject will inhibit growth of a tumor in the subject, and/or result in regression of the tumor. The methods and reagents (e.g., cells) of the invention may also be used prophylactically, to elicit an immune response that decreases a subject's risk of developing a cancer.

30 According to the present invention, an immune response in a subject may be elicited by administration of TRT proteins and fragments and derivatives thereof,

polynucleotides encoding such TRT proteins, fragments, and derivatives, and antigen presenting cells (e.g., dendritic cells) comprising the aforementioned polypeptide and polynucleotide reagents, e.g., dendritic cells displaying TRT antigens in the context of peptide/MHC complexes. The immune response that is evoked may be primarily humoral  
5 (i.e., antibody mediated), primarily cell-mediated (e.g., cytotoxic T-lymphocyte-mediated), or have both humoral and cellular components. Alternatively, T lymphocytes may be activated *ex vivo* and administered to a subject.

Thus, in various aspects of the invention, the immune response to TRT or a TRT-expressing cell is elicited by methods including one or more of the steps of (1)  
10 administering to a subject an antigen presenting cell (APC) that presents a TRT antigenic peptide; (2) administering to a subject T-lymphocytes that recognize a TRT peptide/MHC complex; (3) administering an immunogenic amount of a polynucleotide encoding a TRT protein, polypeptide, or fragment; (4) administering an immunogenic amount of a TRT protein, polypeptide, or fragment. The polynucleotide of (3) or polypeptide of (4) are  
15 usually administered in an amount capable of inducing a Class I MHC-restricted cytotoxic T-lymphocyte response against cells expressing a TRT protein or, alternatively, in an amount capable of inducing the production of antibodies by the subject.

### 20 III. THE USE OF ANTIGEN PRESENTING CELLS (APCs) TO ELICIT AN ANTI-TRT IMMUNE RESPONSE

In one embodiment of the invention, antigen presenting cells (APCs) are used to activate T lymphocytes *in vivo* or *ex vivo*, to elicit an immune response against TRT-expressing cells. APCs are highly specialized cells, including macrophages,  
25 monocytes, and dendritic cells (DCs), that can process antigens and display their peptide fragments on the cell surface together with molecules required for lymphocyte activation. Generally, however, dendritic cells are superior to other antigen presenting cells for inducing a T lymphocyte mediated response (e.g., a primary immune response). DCs may be classified into subgroups, including, e.g., follicular dendritic cells, Langerhans dendritic  
30 cells, and epidermal dendritic cells.

DCs have been shown to be potent simulators of both T helper (Th) and

cytotoxic T lymphocyte (CTL) responses. See Schuler et al., 1997, *Int. Arch. Allergy Immunol.* 112:317-22. *In vivo*, DCs display antigenic peptides in complexes with MHC class I and MHC class II proteins. The loading of MHC class I molecules usually occurs when cytoplasmic proteins (including proteins such as TRT that are ultimately transported to the nucleus) are processed and transported into the secretory compartments containing the MHC class I molecules. MHC Class II proteins are normally loaded *in vivo* following sampling (e.g., by endocytosis) by APCs of the extracellular milieu. DCs migrate to lymphoid organs where they induce proliferation and differentiation of antigen-specific T lymphocytes, *i.e.*, Th cells that recognize the peptide/MHC Class II complex and CTLs that recognize the peptide/MHC Class I complex. An introduction to T lymphocytes and cell mediated immunity is found in Paul, 1993, *FUNDAMENTAL IMMUNOLOGY, THIRD EDITION* Raven Press, New York, NY and the references cited therein.

It is now understood that DCs (or DC precursor cells) can be exposed to antigenic peptide fragments *ex vivo* (referred to as "antigen pulsing"), or genetically modified *ex vivo* to express a desired antigen, and subsequently administered to a patient to induce an anti-antigen immune response. Alternatively, the pulsed or genetically modified DCs can be cultured *ex vivo* with T lymphocytes (e.g., HLA-matched T lymphocytes) to activate those T cells that specific for the selected antigen. Of particular relevance to the present invention, antigen-laden DC may be used to boost host defense against tumors (see, e.g., Hsu, et al., 1996, *Nature Med.* 2:52-58; Young et al., 1996, *J. Exp. Med.* 183:7-11; McArthur et al., 1998, *J. Immunother.* 21:41-47; Tuting et al., 1997, *Eur. J. Immunol.* 27:2702-2707; Nair et al., 1997, *Int. J. Cancer* 70:706-715). It will be appreciated that is not necessary that the target antigen (e.g., target "tumor" antigen) be expressed naturally on the cell surface, because cytoplasmic proteins and nuclear proteins, such as TRT, are normally processed, attached to MHC-encoded products intracellularly, and translocated to the cell surface as a peptide/MHC complex.

In one aspect, the present invention relates to the use of polypeptides and polynucleotides encoding TRT (especially human hTRT), and antigen presenting cells (especially dendritic cells), to elicit an immune response against TRT-expressing cells, such as cancer cells, in a subject. Typically, this involves (1) isolating a hematopoietic stem cells, (2) genetically modifying the cells to express a TRT polypeptide, (3)

differentiating the precursor cells into DCs and (4) administering the DCs to the subject (e.g., human patient). In an alternative embodiment, the process involves (1) isolating DCs (or isolation and differentiation of DC precursor cells) (2) pulsing the cells with TRT peptides, and (3) administering the DCs to the subject. These approaches are discussed in greater detail, *infra*. In a related embodiment, the TRT pulsed or expressing DCs of the invention are used to activate T lymphocytes *ex vivo*. It will be recognized, however, that many variations of each of the procedures described *infra* are known to those of skill (see, e.g., WO97/29182; WO 97/04802; WO 97/22349; WO 96/23060; WO 98/01538; Hsu et al., 1996, *Nature Med.* 2:52-58), and that still other variations may be discovered in the future.

#### A. Genetic Modification of Dendritic Cell Precursors

In one embodiment, DC stem cells are isolated for transduction with a TRT-encoding polynucleotide, and induced to differentiate into dendritic cells. The genetically modified DCs express the TRT polypeptide, and display peptide fragments on the cell surface.

##### (1) Isolation of DC Precursor Cells

Many methods are known for isolating DC precursor cells suitable for transfection with a recombinant TRT-encoding nucleic acid. Human hematopoietic progenitor and stem cells are characterized by the presence of a CD34 surface membrane antigen, which may be used in purification. In one embodiment, for example, human hematopoietic stem cells are obtained by bone marrow aspiration, and the bone marrow mononuclear cells are separated from the other components by means of Ficol density gradient centrifugation and adherence to plastic. The light density, non-adherent cells are obtained and further selected using an anti-CD34 antibody (preferably monoclonal) by standard methods (e.g., incubation of cells with the anti-CD34 antibody, subsequent binding to an immobilized secondary antibody, and removal of nonbound components; see, e.g., Harlow and Lane, 1988, *ANTIBODIES: A LABORATORY MANUAL*, Cold Spring Harbor Laboratory, New York) Alternatively, cells can be obtained by leukapheresis of peripheral blood and anti-CD34 chromatography (see, e.g., Reeves et al, 1996, *Cancer Res.* 56:5672-77).

(2) Genetic Modification of Cells to Express a TRT Polypeptide

In one embodiment of the invention, the DC or DC precursor cell is genetically modified to express a TRT polypeptide (e.g., transduced *ex vivo* with a polynucleotide encoding TRT). Exogenous TRT-encoding polynucleotides may be incorporated into DC as TRT expression cassettes using methods such as those described *infra*. Typically the DC is transformed with an expression cassette comprising a region encoding a TRT polypeptide (or one or more fragments thereof). Upon expression of the TRT expression cassette in the cell, the TRT polypeptide is processed into antigenic peptides expressed on the surface of the DC as complex with MHC class I and II surface molecules. Typically the TRT expression cassette includes an operably linked promoter (to drive expression of the TRT coding sequences). Usually a strong promoter such as a t-RNA pol III promoter, or a pol II promoter with strong constitutive expression is used. Suitable promoters include the constitutive adenovirus major late promoter, the dexamethasone-inducible MMTV promoter, the SV40 promoter, the MRP polIII promoter, the constitutive MPSV promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, and promoter-enhancer combinations known in the art. In alternative embodiments, the TRT coding sequence is introduced into the DC precursor without a linked promoter. In such a case the TRT transcription is directed by an endogenous promoter (e.g., following integration of the TRT coding sequence into the cell chromosome) or a separately introduced promoter (e.g., that becomes linked by recombination). Often the TRT expression cassette is contained in an expression vector such as a plasmid or viral vector, which may also include other elements, e.g., an origin of replication, chromosome integration elements such as retroviral LTRs, and/or selection (e.g., drug resistance) sequences.

In one embodiment all of most (e.g., at least about 60%, at least about 75% or at least about 90%) of the TRT protein is expressed (i.e., coded for) in the TRT expression cassette. In some cases, however, a shorter fragment may be expressed. Usually TRT coding sequence will encode at least about 8, more often 12, still more often at least 30 or at least 50 contiguous TRT amino acid residues.

In some embodiments, the TRT polypeptide expressed has a sequence of a naturally occurring TRT. It will be recognized, however, that the invention is not limited

to naturally occurring sequences. As already noted, fragments of naturally occurring TRT proteins may be used; in addition, the expressed TRT polypeptide may comprise mutations such as deletions, insertions, or amino acid substitutions when compared to a naturally occurring TRT polypeptide, so long as at least one TRT peptide epitope can be processed  
5 by the DC and presented on a MHC class I or II surface molecule. It will be appreciated that it may sometimes be desirable to use TRT sequences other than "wild type," in order to, for example, increase antigenicity of the TRT peptide or to increase TRT peptide expression levels. In some embodiments, the introduced TRT sequences encode TRT variants such as polymorphic variants (e.g., a variant expressed by a particular human  
10 patient) or variants characteristic of a particular cancer (e.g., a cancer in a particular patient).

The TRT expression sequence may be introduced (transduced) into DCs or stem cells in any of a variety of standard methods, including transfection, recombinant vaccinia viruses, adeno-associated viruses (AAVs), and retroviruses (see, e.g., Reeves et al., 1996, *Cancer Res.* 56:5672; Brossart et al., 1997, *J. Immunol.* 158:3270; Ribas et al.,  
15 1997, *Canc. Res.* 57:2865; Carter et al., 1993, WO 93/24641; Kotin, 1994, *Human Gene Therapy* 5:793-801; Specht et al., 1997, *J. Exp. Med.* 186:1213-1221), particle-mediated gene transfer technology (Tuting et al., 1997, *Eur J Immunol.*, 27:2702-2707), or other conventional methods for transforming stem cells are known (e.g., calcium phosphate precipitation; see e.g., Krieger, 1990, *GENE TRANSFER AND EXPRESSION -- A LABORATORY  
20 MANUAL*, Stockton Press, New York, NY; Sambrook et al., 1989, *MOLECULAR CLONING A LABORATORY MANUAL* 2ND ED, Vol. 1-3; and Ausubel et al., eds, 1997, *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, John Wiley & Sons, Inc.). Alternately, TRT-encoding polynucleotide can be packaged into viral particles using packaging cell lines,  
25 which are incubated with the DC stem cells.

### (3) Differentiation of Precursor Cells into DCs

The TRT-peptide expressing hematopoietic progenitor cells described *supra* are induced to differentiate into DCs by conventional methods, e.g., by exposure to  
30 cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF), flt-3 ligand, tumor necrosis factor alpha c-kit ligand (also called steel factor or mast cell factor).

The addition of interleukin-4 (IL-4) to monocyte cultures is reported to help direct cells to develop as dendritic cells, and TNF-alpha, when mixed with undifferentiated stem cells, increases the likelihood that the stem cells will develop as dendritic cells (see Szabolcs et al., 1995, *J. Immunol.* 154:5851-5861). Alternatively, calcium ionophore is used to stimulate the maturation of isolated monocytes into dendritic cells (U.S. Patent 5,643,786).  
5 In one embodiment, DCs are obtained from CD34+ hematopoietic progenitor cells from the blood (e.g., of cancer patients) according to the method described by Bernhard et al., 1995, *Cancer Res.* 55:1099-104. A DC maturation factor may be used to cause "immature DCs" to stably express dendritic cell characteristics (e.g., dendritic cell markers p55 and  
10 CD83; see WO 97/29182). Alternatively, immature DCs may be used to activate T cells (Koch et al., 1995, *J. Immunol.* 155:93-100).

The culture of cells such as those used in conjunction with the present invention, including stem cells and dendritic cells is well known in the art (see, e.g., Freshney, 1994, *CULTURE OF ANIMAL CELLS, A MANUAL OF BASIC TECHNIQUE, THIRD*  
15 *EDITION* Wiley-Liss, New York).

#### (4) Administering the DCs to the Subject (e.g., Human Patient)

The transformed DCs of the invention are introduced into the subject (e.g., human patient) where they induce an immune response. Typically the immune response  
20 includes a CTL response against target cells bearing TRT antigenic peptides (e.g., in a MHC class I/peptide complex). These target cells are typically cancer cells.

When the DCs of the invention are to be administered to a patient, they are preferably isolated from, or derived from precursor cells from, that patient (i.e., the DCs are administered to an autologous patient). However, the cells may be infused into HLA-  
25 matched allogeneic, or HLA-mismatched allogenic patients. In the latter case, immunosuppressive drugs may be administered to the recipient.

The cells are administered in any suitable manner, preferably with a pharmaceutically acceptable carrier (e.g., saline). Usually administration will be intravenous, but intra-articular, intramuscular, intradermal, intraperitoneal, and  
30 subcutaneous routes are also acceptable. Administration (i.e., immunization) may be repeated at time intervals. Infusions of DC may be combined with administration of

cytokines that act to maintain DC number and activity (e.g., GM-CSF, IL-12)

The dose administered to a patient, in the context of the present invention should be sufficient to induce an immune response as detected by assays which measure T cell proliferation, T lymphocyte cytotoxicity, and/or effect a beneficial therapeutic response in the patient over time, e.g., to inhibit growth of cancer cells or result in reduction in the number of cancer cells or the size of a tumor. Typically,  $10^6$  to  $10^9$  or more DCs are infused, if available.

#### B. Peptide Pulsing of Dendritic Cells

In a related embodiment of the invention, DCs are obtained (either from a patient or by *in vitro* differentiation of precursor cells) and pulsed with antigenic peptides having a sequence of TRT (e.g., hTRT). The pulsing results in the presentation of TRT peptides onto the surface MHC molecules of the cells. The TRT-peptide/MHC complexes displayed on the cell surface are capable of inducing a MHC-restricted cytotoxic T-lymphocyte response against target cells expressing TRT polypeptides (e.g., cancer cells).

##### (1) Isolation of DCs

DCs can be obtained by isolating DC precursor cells and inducing them to differentiate into DCs, as described *supra*. Alternatively, DCs may be isolated from both lymphoid and non-lymphoid tissues; typically they are purified from peripheral blood. Methods for isolation of human DCs from blood include apheresis followed by procedures involving density gradient methods, positive selection (e.g., affinity chromatography with antibodies directed to DC surface markers), negative selection, or combinations thereof (see, e.g., WO97/22349; WO95/34638; WO98/01538; WO94/02156).

DC may be isolated from a normal human or from a patient suffering from a disease. In either case, individuals may be treated with colony stimulating factors to increase their number of DC prior to isolation. For example, GM-CSF, may be infused into an individual at  $250 \mu\text{g}/\text{m}^2/\text{day}$  for several days up to three weeks intravenously prior to obtaining the peripheral blood mononuclear leukocytes (PBML) for the purification of DC. This procedure may increase the yield of DC for antigen pulsing and subsequent infusion.

It will be appreciated that, in one embodiment of the invention, "immortalized" or "extended life span dendritic cells" are used. Immortalized DCs may be prepared according to the methods of copending applications USSN 08/912,951 and 08/974,549. In an alternative embodiment, DCs transformed with Epstein-Barr virus (see, 5 WO 97/04802), retroviral vectors containing an oncogene (see, e.g, WO 94/28113) and the like (U.S. Pat. 5,648,219) may be used.

## (2) Pulsing DCs with TRT Peptides

DCs are exposed *ex vivo* to TRT antigens, and allowed to process the 10 antigen so that TRT epitopes are presented on the surface of the cell in the context of a MHC class I (or MHC class II) complex. This procedure is referred to as "antigen pulsing." The "pulsed DCs" may then be used to activate T lymphocytes.

The TRT peptide antigens used for pulsing DCs comprise at least one linear epitopes derived from the TRT protein. TRT proteins or substantial fragments thereof may 15 be used, as they will be taken up and processed by the DCs. Alternatively, short "peptides" may be administered to the DCs.

When TRT peptides are used for pulsing, they will usually have at least about 6 or 8 amino acids and fewer than about 30 amino acids or fewer than about 50 amino acid residues in length. In one embodiment, the immunogenic TRT peptide has 20 between about 8 and 12 amino acids. A mixture of hTRT protein fragments may be used; alternatively a particular peptide of defined sequence may be used. The TRT peptide antigens may be produced by *de novo* peptide synthesis, enzymatic digestion of purified or recombinant hTRT, by purification of telomerase from a natural source (e.g., a patient or tumor cells from a patient), or expression of a recombinant polynucleotide encoding a 25 hTRT fragment.

It has been found that the various class I MHC alleles bind peptides having, from N-terminus to C-terminus, first, second and/or third conserved amino acid residues separated by a certain number of amino acids. The binding motifs for several human HLA-A alleles are provided in WO 94/03205. Thus, in one embodiment of the invention, 30 the TRT fragment or peptide comprises at least one linear TRT epitope having a class I MHC binding motif, and, in particular, an HLA-A1 binding motif, an HLA-A2.1 binding

motif, an HLA-A3.2 binding motif, an HLA-A11 binding motif, an HLA-A24.1 binding motif or an HLA-B7 binding motif. Provided with the nucleic acid and protein sequences of a TRT, such as the hTRT sequences provided in Figure 1 and Figure 2, it is within the ability of one of ordinary skill to identify and produce various large or small polypeptides or peptides that comprise particular HLA binding motifs. If desired, a mixture of TRT peptides may be used, so that a wide variety of HLA binding motifs are represented.

The amount of TRT antigen used for pulsing DC will depend on the nature, size and purity of the peptide or polypeptide. Typically, from about 0.05 µg/ml to about 1 mg/ml, most often from about 1 to about 100 µg/ml of TRT peptide is used. After adding the TRT peptide antigen(s) to the cultured DC, the cells are then allowed sufficient time to take up and process the antigen and express antigen peptides on the cell surface in association with either class I or class II MHC. Typically this occurs in about 18-30 hours, most often about 24 hours. In one exemplary embodiment enriched DC are resuspended ( $10^6$  cells/ml) in RPMI media (Gibco) and cultured with (50 µg/ml) hTRT peptide antigens overnight under standard conditions (e.g., 37°C humidified incubator/5% CO<sub>2</sub>).

### (3) Administering the DCs to the Subject

The pulsed DC are washed in physiologic saline and administered to a subject as described *supra*, Section (A)(4).

### C) Administration of TRT-Reactive T Lymphocytes

Antigen-specific T lymphocytes may be prepared *ex vivo* by collecting naive T lymphocytes from an individual (e.g., CD8+ T lymphocytes), contacting them with the DCs of the invention (e.g., TRT transformed or pulsed DCs) so that TRT-reactive T lymphocytes proliferate. In an alternative embodiment, the DCs are pulsed with TRT antigens at the time of co-culture with T lymphocytes. The expanded TRT-reactive T lymphocyte population may then be administered to the individual, or may be used for *in vitro* assays. When administered to a patient, the *ex vivo* activated-T lymphocytes are cytotoxic against target cells bearing TRT antigenic peptides, such as cancer cells (see, e.g., WO 94/ 02156).

Several techniques are known for isolating T lymphocytes. In one method, Ficoll-Hypaque density gradient centrifugation is used to separate PBMC from red blood cells and neutrophils according to established procedures. Cells are washed with AIM-V (GIBCO) supplemented with 2 mM glutamine, 10  $\mu$ g/ml gentamicin sulfate, 50  $\mu$ g/ml streptomycin and 1% fetal bovine serum). Enrichment for T lymphocytes is performed by negative or positive selection with appropriate monoclonal antibodies (e.g., anti-CD2, CD3, CD4, CD5 and CD8) coupled to columns or magnetic beads according to standard techniques. Alternatively, fluorescence-activated cell sorting is used. An aliquot of cells is analyzed for cell surface phenotype including CD4, CD8, CD3 and CD14. Cells are washed and resuspended at a concentration of  $5 \times 10^5$  cells per ml of AIM-V supplemented as above and containing 5% FBS and 100 U/ml recombinant IL-2. The T lymphocytes are then cultured with pulsed or genetically modified DCs, optionally in the presence of low doses of IL-4 and IL-2.

The activated T lymphocytes can then be administered to a subject (e.g., infused) as described for DCs, *supra*. The expanded T lymphocytes may be administered alone, or in combination with lymphokines such as IL-2 and/or IL-4.

#### IV. IN VITRO ASSAYS

The present invention provides commercially valuable assays, e.g., for identifying TRT-expressing cells. In the assays of the invention, dendritic cells are transformed with a TRT-encoding nucleic acid or pulsed with a TRT peptide. The DC is used to activate isolated T lymphocytes, which are then tested for cytotoxic activity against a class of cells thought to express TRT. Cytotoxicity (e.g., as indicated in a standard assays such as a  $^{51}\text{Cr}$  release assay, *infra*) indicates that the cells express TRT is amounts sufficient to mediate a T lymphocyte recognition of the cell. This provides investigators with an assay for TRT-expressing cells.

To demonstrate that CTL have cytolytic activity against a cell (e.g., a cell expressing TRT, such as a cancer cell) the target antigen-presenting cells and effector CTL cells are mixed in culture and target cell lysis is observed. Any suitable method for measuring cell lysis can be used by one skilled in the art. For example, a radioactivity release assay can be used to measure lysis of the target cells. For example, the target cells

are labeled with radioactive reagents such as  $^{51}\text{Cr}$ , which are taken up by live cells. Following labeling, the target cells are washed and mixed with specific CTLs. Supernatants are harvested after a suitable time and counted to determine the percent radioactivity release. Other methods to determine the amount of cell lysis include trypan  
5 blue exclusion, in which living cells that exclude the dye are counted and compared to a control sample of non-presenting cells treated in the same manner.

\*\*\*

10 All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent document were so individually denoted.

WHAT IS CLAIMED IS:

1. A method of activating a T lymphocyte, comprising contacting the T lymphocyte with a dendritic cell that expresses a telomerase reverse transcriptase (TRT) polypeptide encoded by a recombinant nucleic acid.  
5
2. The method of claim 1, wherein the TRT polypeptide is a human TRT (hTRT) polypeptide.
3. The method of claim 2, wherein the hTRT polypeptide is has the sequence  
10 of set forth in Figure 1, or a subsequence thereof.
4. The method of claim 2 wherein the hTRT polypeptide is full-length.
5. The method of claim 1, wherein the dendritic cell contacts the T  
15 lymphocyte *in vivo*.
6. The method of claim 1, wherein the dendritic cell contacts the T lymphocyte *in vitro*.
7. The method of claim 1, wherein the dendritic cell is a human cell.  
20
8. A recombinant dendritic cell which comprises a recombinant TRT expression cassette.
9. A pharmaceutical composition comprising the dendritic cell of claim 9 and  
25 a pharmaceutically acceptable carrier.
10. The recombinant dendritic cell of claim 8, wherein the recombinant expression cassette was transduced into a stem cell, which stem cell was then differentiated  
30 into the dendritic cell.

11. The method of claim 10, wherein the stem cell is differentiated into a dendritic cell *in vitro*.

5 12. A method of eliciting an immune response in a human patient comprising:  
(a) obtaining human dendritic cells;  
(b) transducing a TRT expression cassette into the cells; such that they are capable of expressing a hTRT polypeptide;  
(c) administering the cells to the human patient.

10 13. The method of claim 12, wherein the dendritic cells are isolated from the human patient.

14. The method of claim 13, wherein the dendritic cells are obtained from hematopoietic precursor cells.

15 15. A method of eliciting an immune response in a human patient comprising:  
(a) obtaining human dendritic cells;  
(b) pulsing the cells with a hTRT antigen;  
(c) administering the cells pulsed with the hTRT antigen to the human  
20 patient.

16. The method of claim 15, wherein the dendritic cells are isolated from the human patient.

25 17. The method of claim 15, wherein the cells are pulsed with one or more hTRT antigenic peptides, wherein said hTRT antigenic peptides are less than 50 amino acid residues in length.

30 18. A method for identifying a cell expressing hTRT comprising:  
transducing a dendritic cell with a recombinant expression cassette comprising a nucleic acid encoding a hTRT polypeptide;

contacting a T lymphocyte with the transduced dendritic cell, thereby providing an activated T lymphocyte;

contacting a target cell with the activated T lymphocyte; and,

monitoring the effect of the activated T lymphocyte on the target cell.

5

MPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQGWRLVQRGDP  
AAFRALVAQCLVCVPWDARPPPAAPSFQVSCLELVARVLQRL  
CERGAKNVLAFGFALLDGARGGPPPEAFTTSVRSYLPNTVTDALR  
GSGAWGLLLRRVGGDDVLVHLLARCALFVLVAPSCAYQVCGPPLY  
QLGAATQARPPPHASGPRRRLGCERAWNHSVREAGVPLGLPAPG  
ARRRGGSASRSLPLPKRPRRGAAPEPERTPVGQGSWAHPGRTRG  
PSDRGFCVVSPARPAEEATSLEGALSGTRHSHPSVGRQHHAGPP  
STSRPPRPWDTPCPPVYAETKHFYSSGDKEQLRPSFLLSSLRP  
SLTGARRLVETIFLGSRPWMPGTFRRLPRLPQRYWQMRPLFLEL  
LGNHAQCPYGVLLKTHCPLRAAVTPAAGVCAREKPQGSVAAPPE  
EDTDPRRLVQLLRQHSSPWQVYGFVRACLRRLVPPGLWGSRHNE  
RRFLRNTKKFISLGKHAKLSLQELTWKMSVRDCAWLRRSPGVGC  
VPAAEHLRLREEILAKFLHWLMSVYVVELLRSFFYVTETTFQKNR  
LFFYRKSWSKLQSIGIRQHLKRVQLRELSEAEVRQHREARPAL  
LTSRLRFIPKPDGLRPIVNMDYVVGARTFRREKRAERLTSRVKA  
LFSVLNYERARRPGLLGASVLGLDDIHRAWRTFVLRVRAQDPPP  
ELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYAVVQ  
KAAHGHVRKAFKSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVI  
EQSSSLNEASSGLFDVFLRFMCHHAVRIRGKSYVQCQGIPOGSI  
LSTLLCSLCYGD MENKLFAGIRRDGLLLRLVDDFLLVTPHLTHA  
KTFLRTLVRGVPEYGCVVNLRKTVVNFVEDEALGGTAFVQMPA  
HGLFPWCGLLLDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGR  
NMRRKLFGLVRLKCHSLFLDLQVNSLQTVCTNIYKILLQAYRF  
HACVLQLPFHQVWKNPTFFLRVISDTASLCYSILKAKNAGMSL  
GAKGAAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSRLTAQ  
TQLSRKLPGTTLTALEAAANPALPSDFKTILD

FIG. 1

```

1 gcagcgctgc gtcctgctgc gcacgtggga agccctggcc ccggccaccc ccgcgatgcc
61 ggcgctcccc cgctgcccag ccgtgcccgc cctgctgccc agccactacc gcgaggtgct
121 gccgctggcc acgttcctgc ggcgctgggg gcccaggggc tggcggtggg tcgagcgccg
181 ggaccggcg gctttccgcg cgctggtggc ccagtgcctg gtgtgctgac cctgggacgc
241 acggccgccc cccgcccggc cctccttcgg ccaggtgtcc tgcctgaagg agctggtggc
301 ccgagtgctg cagaggctgt ggggcccggc ggggcccccc cgaggccttc accaccagcg tggcttcgc
361 gctgctggac cctgcccac acggtgaccg acgactgccc ggggagcggg gcgtgggggc tgctgctgcg
421 cctgcccac acggtgaccg acgactgccc ggggagcggg gcgtgggggc tgctgctgcg
481 ccgctgccc acggtgaccg acgactgccc ggggagcggg gcgtgggggc tgctgctgcg
541 ggctcccagc tgcgctacc aggtgtgccc gccgcccgtg taccagctcg gcgtgcccac
601 tcaggcccgg ccccggcac acgctagtgg accccgaagg cgtctgggat gcgaacgggc
661 ctggaacat agcgtcaggg aggcgggggt cccctggggc ctgcccagccc cgggtgcccag
721 gagggcgggg ggcagtgcca gccgaagtct gccgttgccc aagaggccca ggcgtggcg
781 tgcccctgag ccggagcgga cgcctgtgg gcaggggtcc tggggccacc cgggcaggac
841 gctgtggacc agtgaccgtg gtttctgtgt ggtgtcacct gccagaccgg ccgaagaagc
901 cactctcttg gaggtgctgc tctctggcac gcgcccactcc caccatccg tgggcccgca
961 gcaccacggc ggcggggcat ccacatcgcg ccaccacgt ccctgggaca cgccttgtcc
1021 cccgggtgat gccgagacca ggcacttccc ctactcctca ggcgctcgga ggctcgaggc
1081 gccctccttc ctactcagct ctctgaggcc cagcctgact ggcagggact ccccgccagg tggcccgcct
1141 gaccatcttt ctgggttcca ggcctgggat gccagggact ccccgccagg ctgcttggga accacgcgca
1201 gcccagcgcc tactggcaaa tgcggcccct gtttctggag ctgcttggga accacgcgca
1261 gtgcccctac ggggtgctcc tgggcccggg tgggcccggg cgagctgccc tcaaccccagc
1321 agccgggtgc tgggcccggg tgggcccggg tgggcccggg agggaggagg ggaggtgta
1381 cacagacccc cgtcgccctg tgcagctgct ccgcccagcac agcagcccc tggcagggca
1441 cggcttcctg cgggcccggc tgcgcccggc ggtgccccca ggcctctggg gctccaggca
1501 caacgaacgc cgcttcccac ggaacaccaa gaagtccatc tccctgggga agctgcccac
1561 gctctcctg caggagctga cgtggaagat gagcgtgccc gactgcccct ggctgcccag
1621 gagcccaggg gttggctgtg ttccggccgc agagcaccgt ctgctgagg agatcctggc
1681 caagttcctg cactggctga tgagtggtg cgtcgtcgag ctgctcagg ctctcttta
1741 tgcacggag accacgttcc aaaagaacag gctcttttcc taccggaaga gtgtctggag
1801 caagttgcaa agcattggaa tcagacagca cttgaaggag gtgcagctgc gggagctgtc
1861 ggaagcagag gtcagggcag atcgggaagc cagggcccgc ctgtgacgt ccagactccg
1921 cttcatcccc aagcctgacg ggctgcccgc gattgtgaac atggactacg tcgtgggagc
1981 agaacggttc cgcagagaaa agaggggccc gcgtctcacc tcgagggtga aggcactgtt
2041 cagcgtgctc aactacgagc gggcgcccgc ccccgcccct ctggggcgcct ctgtgctggg
2101 cctggacgat atccacaggg cctggcgcac cttcgtgctg cgtgtgcccg cccaggaccc
2161 gccgctgag ctgtactttg tcaagggtga tgtgacgggc cgtacggaca ccatcccaca
2221 ggacaggttc cgcagaggtc tgcgcccagc catcaaaccc cagaacacgt actgctgccc
2281 tcgggtatgc gtggtccaga aggcggccca tgggcacgtc cgcaaggcct tcaaggacca
2341 cgtctctacc ttgacagacc tccagccgta catgagacag ttcgtggctc acctgcagg
2401 gaccagcccc ctgagggatg ccgctgctat cgagcagagc tcctccctga atgagggcag
2461 cagtgccctc ttcgacgtct tccacgctt catgtgccac cagcggctgc gcatcagggg
2521 caagtcctac gtccagtgcc aggggatccc gcagggctcc atcctctcca cgctgctctg
2581 cagcctgtgc tacggcgaca tggagaacaa gctgtttgcg gggattcggc gggacgggct
2641 gctcctgctg ttgggtggat atttctgtt ggtgacacct cacctcacc cagcgaacac
2701 cttcctcagg accctgggtc gaggtgtccc tgagtatggc tgcgtggtga acttgcggaa
2761 gacagtggtg aacttcccct tagaagacga ggccctgggt ggcacggctt ttgttcagat
2821 gccggcccac ggcctattcc cctggtgccc cctgctgctg gatacccgga cctggagggt
2881 gcagagcgac tactccagct atgcccggac ctccatcaga gccagtctca cctcaaccg
2941 cggcttcaag gctgggagga acatgcgtcg caaactcttt ggggtcttgc ggctgaagtg
3001 tcacagcctg tttctggatt tgcaggtgaa cagcctccag acggtgtgca ccaacatcta
3061 caagatcctc ctgctgcagg cgtacaggtt tcacgcatgt gttgtgagc tcccatttca
3121 tcagcaagtt tggagaacac ccacattttt cctgcccgtc atctctgaca cggcctccct
3181 ctgctactcc atcctgaaag ccaagaacgc agggatgtcg ctgggggcca agggcgccgc
3241 cggccctctg ccctccaggc ccgtgcagtg gctgtgccac caagatttcc tgctcaagct
3301 gactcgacac cgtgtcacct acgtgccact cctgggggtc ctcaggacag cccagacgca
3361 gctgagtcgg aagctcccgg ggacgacgct gactgccctg gagcccgag ccaacccggc
3421 actgcccctc gacttcaaga ccactctgga ctgatggcca cccggccaca gccagggcga
3481 gagcagacac cagcagccct gtcacgcccg gctctacgtc ccagggaggg agggggcgcc
3541 cacaccagg cccgcaccgc tgggagctcg aggcctgagt gagtgtttgg ccgagggctg
3601 catgtccggc tgaaggctga ggtgcccgtg gaggcctgag cgagtgtcca gccaagggtc
3661 gagtgtccag cacacctgcc gtcttcaatt cccacaggc tggcgctcgg ctccacccca
3721 gggccagctt ttccctacca ggagcccggc ttccactccc cacataggaa tagtccatcc
3781 ccagattcgc cattgttcac cctcgccctt gccttccacc cccacatcc ccaaggtgtg
3841 aggtggagac cctgagaagg accctgggag ctctgggaat ttggagtgac caaaggtgtg
3901 ccctgtacac aggcgaggac cctgcacctg gatgggggtc cctgtgggtc aaattggggg
3961 gaggtgctgt gggagtaaaa tactgaatat atgagttttt cagttttgaa aaaaa

```

FIG. 2

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/06898

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 5/06, 5/22, 9/00

US CL : 435/29, 372, 377

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/29, 372, 377

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NORRBACK, K.-F. et al. Teleomeres and telomerase in normal and malignant hematopoietic cells. Eur. J. Cancer. 1997, Vol. 33, No. 5, pages 774-780, see entire document.	1-18
Y	GREAVES, M. Is telomerase activity in cancer due to selection of stem cells and differentiation arrest? Trends Genet. April 1996, Vol. 12, No. 4, pages 127-128, see entire document.	1-18
Y,P	YASUI, W. et al. Expression of telomerase catalytic component, telomerase reverse transcriptase, in human gastric carcinomas. Jpn. J. Cancer Res. November 1998, Vol. 89, pages 1099-1103, see entire document.	1-18

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*g*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

20 JUNE 1999

Date of mailing of the international search report

14 JUL 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

MARY TUNG

Telephone No. (703)308-0196

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/06898

### B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

MEDLINE, CANCERLIT, SCISEARCH, BIOSIS, ELSEVIER, JPO, EPO, USPAT

telomerase, reverse transcriptase, dendritic cell, langerhans cell, antigen presenting cell, APC, Kupffer cell, stem cell

progenitor cell, precursor cell, hematopoietic cell, recombinant, vector, transfection, hTERT